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DETERMINATION OF FOOD PREFERENCE OF *STENTOR COERULEUS*

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Schaeffer (1910) found that the ciliate protozoön, *Stentor coeruleus,* selected among the particles that were brought to its buccal cavity (by its adoral membranelles). Some particles were preferentially rejected by a localized ciliary reversal while others were carried to the cytostome and ingested. Selection was hypothesized not only among the particles reaching its buccal cavity successively, but also among particles reaching the cavity at the same time. Furthermore, the amount ingested depended upon the other substances present. For example, Schaeffer found that carmine particles, although indigestible, were ingested by stentors in the absence of food organisms, but rarely taken when food organisms were present. Stentors were found to discriminate more perfectly when almost satiated than when very hungry, since hungry individuals ingested particles such as carmine and india ink. Schaeffer found that this species also discriminated between different types of organisms, ingesting some (*Euglena* sp., *Phacus triqueter*) with great readiness, while others (*Trachelomonas hispida, Phacus longicaudus*) were rarely ingested.

Hetherington (1932) reported that on the basis of an extensive series of trials, *S. coeruleus* ingested various autotrophs "sparingly" when hungry. The autotrophs tested included *Gonium pectorale,* several species of *Euglena, Trachelomonas,* and diatoms. In contrast, stentors were found to ingest "avidly" many of the ciliates tested. Hetherington suggested that *S. coeruleus* had a "general preference" for ciliates and was capable of selection even within this group. Tartar (1961) reviewed these studies as well as others and concluded that "on the evidence, food selection does occur in *Stentor,* though by no means perfect and distinctly related in its acuity to the state of the organism."

Such studies, however suggestive, fail to account for differences in "prey catchability" or provide a measure of the statistical significance of preferences. The work reported here makes use of a new definition of food preference (Rapport and Turner, 1970) which lends itself to a determination of preference in the predator-prey context without confounding differences in prey catchability. The method involves comparing the mean number of prey consumed when each prey species is present alone with the mean number of prey of each species consumed when several species are present at the same time.

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Materials and Methods

Stentor coeruleus was collected from Grenadier Pond, Toronto, Ontario and a clone was established for the purpose of these experiments. It was maintained according to culture methods used by Tartar (1961). Eighteen to 24 hours prior to the experiment, stentors were removed from culture and placed in new cultures

Table I

Mean number of prey consumed per stentor in single and mixed culture feedings. Tetrahymena pyriformis (*T*), Chilomonas paramecium (*C.p.*), Euglena gracilis (*E*), *and* Chlamydomonas reinhardti (*C.r.*)

Prey species	Replicate											Mean
	1	2	3	4	5	6	7	8	9	10	11	
C.p.	16.44	14.24	19.48	25.09	31.26	N.A.*	22.88	14.64	26.44	14.04	12.39	19.69
C.r.	47.32	45.60	58.64	67.88	86.92	283.04	182.64	149.92	153.04	76.78	52.52	109.50
E	22.04	12.28	10.76	27.84	18.52	14.42	9.60	11.44	6.28	5.16	11.74	13.64
T	9.76	4.92	11.56	12.40	13.00	11.18	10.96	17.48	14.84	5.86	6.60	10.77
C.r. with C.p.	27.96	30.76	49.48	57.68	91.12	114.50	61.88	64.44	34.08	23.32	97.86	59.37
	10.48	20.00	20.48	21.08	31.92	28.18	12.00	40.92	13.52	10.48	30.23	21.75
C.r. with E	49.16	49.68	39.60	34.12	139.56	87.76	158.16	94.72	40.50	29.96	22.16	67.76
	9.32	15.44	5.60	8.12	10.40	20.16	18.28	3.44	3.13	6.70	17.08	10.69
C.r. with T	7.68	23.08	12.00	12.80	20.12	110.76	44.08	14.76	29.76	6.43	10.20	26.51
	8.36	12.52	9.00	4.12	12.52	16.36	18.67	5.60	17.40	17.64	11.40	12.14
C.p. with E	13.68	13.04	16.52	23.16	31.44	11.88	33.64	22.08	5.92	22.43	8.26	18.36
	8.04	2.52	4.20	4.72	8.40	16.92	10.68	2.84	2.36	5.43	3.00	6.28
C.p. with T	12.08	12.16	13.20	13.76	11.84	22.92	12.08	41.09	13.72	5.00	8.48	15.12
	8.76	6.04	5.00	6.16	4.80	12.44	7.20	17.73	7.88	1.33	1.38	7.15
E with T	4.56	15.64	2.16	9.76	6.04	10.65	0.36	5.20	7.00	4.89	20.72	7.90
	6.32	10.76	7.24	15.96	10.24	9.35	3.80	16.60	7.26	9.11	8.92	9.59

* Not available due to inadequate fixation.

with reduced levels of prey organisms which served to induce a level of starvation which insured an adequate feeding response to prey offered during the experiment.

After stentors were washed gently 3 times in millipore-filtered pond water (0.2 μ pore) to remove bacteria and other organisms which serve as food in cultures, they were then transferred in one ml of millipore-filtered pond water to an embryological block cell. Solid embryological block cells of 4 ml capacity with a true hemispherical cavity were obtained from P. K. Dutt and Co. Ltd.,

Bromley, Kent, England. Prey species were added and stentors were allowed to feed for a period of twenty minutes. The choice of feeding period (20 minutes) enabled most stentors to capture prey but not digest them to the stage where prey recognition becomes difficult. Subsequently, stentors were washed 3 times in sterile pond water to remove adhering prey and then were fixed in a weak solution of formalin. Several drops of 6% formalin were added to 10 ml of millipore-filtered pond water containing washed stentors. Stentors in this state remained well preserved for several weeks. During this period, each stentor was examined individually using light microscopy and the number of each prey species ingested was noted. As food vacuoles containing prey often were obscured by each other it was necessary to compress a stentor gently under a coverglass in order to count all prey present.

Four prey species, *Tetrahymena pyriformis,* strain GL, *Euglena gracilis, Chilomonas paramecium* and *Chlamydomonas reinhardti,* representing protistan genera commonly in fresh water ponds in which *S. coeruleus* can be found, were used in these experiments. Prey were washed several times by alternative gentle centrifugation and resuspension in millipore-filtered pond water and subsequently examined microscopically for damage due to washing. The following prey densities were found to be appropriate for the preference tests: *Tetrahymena pyriformis* 10,000/ml, *Euglena gracilis* 15,000/ml, *Chilomonas paramecium* 30,000/ml, *Chlamydomonas reinhardti* 60,000/ml. These densities approximated the "standard densities" required to determine predator food preferences (Rapport and Turner, 1970). Standard density is defined as the minimum density of prey such that the predator would be able to fulfill its food requirements from any single species alone in the mixed prey environment.

The axenic cultures of prey organisms were obtained from the following sources: *Tetrahymena pyriformis* from Joel Hermolin, University of Toronto, *Euglena gracilis* from L. Cohen, York University, *Chlamydomonas reinhardti* from E. Rapport, York University, and *Chilomonas paramecium* from H. S. Ducoff, University of Illinois.

The experimental design consisted of 11 treatments and 11 replicates with a sample size of 25 stentors in each treatment consisting of either zero (control), one, or two prey species, each in their standard density. The entire experiment was carried out in a controlled temperature room at $18 \pm 1°$ C.

In order to calculate preference, one must have an estimate of μ_1, the mean number of prey species 1 consumed in a standard time interval in single culture; μ_2, the mean number of prey species 2 consumed in single culture; μ, the mean number of prey consumed in mixed culture; $\mu_1{}^*$, the mean number of prey species 1 consumed in mixed culture and $\mu_2{}^*$, the mean number of prey species 2 consumed in mixed culture. In the absence of preference, the predator achieves its food requirements half from prey species 1 and half from prey species 2. Thus:

$$\mu = \frac{\mu_1}{2} + \frac{\mu_2}{2}.$$

If preferences are exercised, the mean number of mixd prey taken can be written:

$$\mu = \frac{p_1\mu_1}{2} + \frac{p_2\mu_2}{2}$$

The parameters p_1 and p_2 are preference coefficients and can be computed as follows:

$$p_1 = \frac{2\mu_1^*}{\mu_1}; \quad p_2 = \frac{2\mu_2^*}{\mu_2}$$

The relative preference $p_{1,2}$ denotes preference for prey species 1 if its value is positive and prey species 2 if negative. It is defined by: $p_{1,2} = p_1 - p_2$.

Observations and Results

The mean prey consumption data are given in Table I. It can be seen that the mean consumption differed among the prey species, ranging from 10.7 in the case of *Tetrahymena pyriformis* to 109.5 for *Chlamydomonas reinhardti*. This difference reflects both the difference in average size of the prey species (as shown

Table II

Relative preference ($p_{1,2}$) of Stentor coeruleus *for selected pairs of prey species*

Prey species		Relative preference Replicate #										
(1)	(2)	1	2	3	4	5	6	7	8	9	10	11
T	C.r.	1.4	4.1	1.1	0.3	1.5	2.1	2.9	0.5	2.0	5.9	3.1
T	E	0.9	1.8	0.8	1.9	0.9	0.2	0.6	1.0	−1.2	1.2	−0.8
C.p.	C.r.	0.1	1.5	0.4	0.0	0.0	N.A.*	0.4	4.7	0.6	0.9	1.1
C.p.	E	0.9	1.4	0.9	1.5	1.1	N.A.*	0.7	2.5	−0.3	1.1	0.8
C.p.	T	−0.3	−0.7	0.5	0.1	0.0	N.A.*	−0.3	3.6	0.0	0.3	1.0
C.r.	E	1.2	−0.3	0.3	0.4	2.1	−2.2	−2.1	0.7	−0.5	−1.8	−2.1

* Not available due to inadequate fixation.

in Table V) and the difference in the "standard" density appropriate for each prey species. The variance between replicates demonstrates the need for a large number of replicates in order to obtain statistically significant results. In Table II, the relative preference values are given as calculated from the basic data in Table I according to the methods described above. In the absence of preference and allowing for sources of variance, we would expect that on average, an equal number of preference coefficients would be positive and negative. Significant deviations from the no-preference case were observed in all cases in which algal and non-algal prey were paired.

The results of the statistical analysis of these data are shown in Table III. It is apparent that *S. coeruleus* prefers *Chilomonas paramecium* when paired with either *Chlamydomonas reinhardti* or *Euglena gracilis*, and prefers *Tetrahymena pyriformis* to *Chlamydomonas* and *Euglena*. (Although P exceeds 0.05 in the case where *Tetrahymena* was paired with *Euglena*.)

In contrast there was no evidence of significant preferences between *Chilomonas* and *Tetrahymena*, or *Euglena* and *Chlamydomonas*.

Table IV presents the data on average consumption in mixed prey feedings as a percentage of the average consumption in single prey feedings. In each case,

TABLE III

Food choice of Stentor coeruleus *when two prey species are simultaneously available.* Tetrahymena pyriformis (*T*), Chilomonas paramecium (*C.p.*), Euglena gracilis (*E*) *and* Chlamydomonas reinhardti (*C.r.*) *were used as prey. Each replicate consisted of three groups of 25 stentors. One group was fed both prey simultaneously, while the other two groups were fed single prey species*

Prey species		Number of replicates	Number of replicates in which prey 1 preferred	Probability*
(1)	(2)			
T	C.r.	11	11	0.001
T	E	11	9	0.07
C.p.	C.r.	10**	9	0.02
C.p.	E	10**	9	0.02
C.p.	T	10**	6	N.S.***
C.r.	E	11	5	N.S.***

* Probability of obtaining results by chance alone if no real preference exists. These probabilities were calculated as the exact probability of obtaining no more than the percentage of minority results assuming that the distribution of relative preference is binomial and that preference could be for either prey. The calculation is made by summing the appropriate number of terms of the binomial expansion. Similar results were obtained by use of T-test statistics.

** Data from one replicate was not obtained due to inadequate fixation.

*** N.S. Not Significant. Probability of obtaining results by chance >0.10.

stentors reduced their consumption of the non-preferred species by a greater amount than the preferred species. These data show that the population of stentors did discriminate between algal and non-algal prey species. When non-algal prey were paired with algal prey in mixed culture, stentors took approximately the same quantity of the non-algal prey consumed in single culture, while taking only one-quarter to one-half the amount of the algal prey consumed in single culture. When the two algal species were paired, or when the two non-algal species were paired, stentors consumed about three-quarters of the quantities of each prey species consumed in single culture (within the limits of experimental error). Thus although the stentors increased its total prey consumption by approximately 50% in the mixed-prey feedings, the increase favored the consumption of non-algal

TABLE IV

Average prey consumption in mixed prey feeding as percentage of single prey feeding consumption

	Euglena	*Tetrahymena*	*Chlamydomonas*	*Chilomonas*
*Euglena**	—	57%	78%	46%
Tetrahymena	89%	—	112%	66%
Chlamydomonas	61%	24%	—	54%
Chilomonas	93%	76%	110%	—

* Reference species are shown in the 1st column. For example, in the mixed feeding of *Euglena* and *Tetrahymena*, stentors consumed 57% of the number of euglenae taken in single culture.

prey when paired with algal prey, while being drawn more equally from both populations when algal prey or non-algal prey were paired.

The increase in the amount consumed when both prey are present may be attributed to an increase in total prey density suggesting that the prey densities chosen for the experiment were somewhat below the "standard density." Thus stentors may have consumed some non-preferred prey in part because they were not fully satiated with the quantities of preferred prey that they could capture. It is also possible that there is a high cost of sorting out the non-preferred prey in cases where both prey are simultaneously brought to the "selection site."

Discussion

Our findings are consistent with Hetherington's speculation that *Stentor coeruleus* has definite preferences for non-algal prey over algal prey. Considering all four prey species, *S. coeruleus* demonstrates a remarkably consistent preference pattern.

Table V

Size ranges of species used as prey

Prey	Length (μ)
Tetrahymena pyriformis (Corliss, 1953)	34–74
Euglena gracilis (Gojdics, 1953)	31–53
Chilomonas paramecium (Kudo, 1966)	30–40
Chlamydomonas reinhardti (Levine, 1960)	8–15

As Schaeffer (1910) has indicated, the degree of preference may vary in part with the hunger state of *S. coeruleus*. This factor may account for some of the variance in the relative preference values obtained. We attempted to control the hunger state of the predator by placing all stentors in a standardized feeding condition for 24 hours prior to the beginning of the experiment. However, since the first and last replicates were done approximately 14 hours apart, hunger states may have indeed varied between replicates. Other factors such as cell cycle may have also affected their feeding response.

It is of interest to note that preferences were not correlated with differences in prey densities used in the experiment, nor were they correlated with differences in prey size as shown in Table V.

The existence of food preferences at the protozoan level has been documented for *Stentor coeruleus*. Using other definitions, food preferences have been reported for many "higher" organisms, both invertebrates and vertebrates (Murdoch 1969, Thompson, 1965). To the extent that food preferences are found in all animal phyla, food preferences would appear to be of fundamental adaptive significance for organisms. To the extent such preferences correlate with the "welfare" of the predator, preference may explain "predator switching" and changes in predator strategies from energy maximizers to time minimizers (Rapport, 1971).

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Summary

Four protistan species, *Tetrahymena pyriformis, Chilomonas paramecium, Euglena gracilis,* and *Chlamydomonas reinhardti,* were fed individually or in pairs to the ciliate *Stentor coeruleus.* Making use of a new definition of food preference which does not confound catchability with choice, this species' food preferences were measured by comparing the mean consumption of a population of stentors when each prey species was present alone with the mean consumption when a pair of prey species was present. *S. coeruleus* was found to exhibit consistent food preferences, preferring protozoan to algal prey while indicating no preference when choosing between algal or between protozoan prey. Preferences were not correlated with prey size.

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